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Introduction

There is an increasing interest in analysis of blood samples for searching some doping substances which are difficult, or impossible, to detect during the classical urine testing .
The experimental administration of AAS to healthy humans can not be applied because of ethical and legal regulations. However, it is a common practice that same of sportsmen, especially body builders, voluntarily use these substances for enhancement strength and power. The abusing of AAS causes some health hazard by development cardiovascular diseases and would even bring a sudden death to the sportsmen. The latter case has already happened in Poland.
In prevention action against AAS use by sportsmen exercising in local clubs the Sport Medicine Outpatients Clinic of the Institute of Sport offered to group of anonymous body builders a free medical examination and full information about their state of health and about the negative consequences of AAS application. The subjects, using voluntarily AAS, trained for themselves and were not body building competitors. They were informed on potential adverse effects of AAS on their health and informed consent was obtained from all of the subjects. The study, without any involvement of drug application to the subjects, was approved by the Ethical Commission of the Institute of Sport.

The aim of the present study was to estimate the diagnostic validity of high density lipoproteins-cholesterol, creatinine kinase and luteinizing hormone measured in blood serum, in 17 body builders currently taking AAS (on cycle) and in 13 body builders who stopped using the AAS (off cycle).

Material and Methods

Blood samples were taken from the subjects during medical examination at the Sport Medicine Outpatients Clinic of the Institute of Sport in Warsaw. After 12 h fasting venous blood was drawn from the anterior cubical fossa, while the subjects rested in seating position. The blood samples were also taken after an incremental exercise performed for determination of the subjects' $VO2_{max}$. Urine samples were collected at the morning and analysed in the Department of Antidoping Research of the Institute of Sport. Venous blood samples were immediately centrifuged and the serum, divided by three portions, was stored at deep freezer (-70^0C). High density lipoproteins-cholesterol (HDL-C) were measured by a phosphotungstic, and magnesium chloride precipitation with a commercial kits (Analco, Poland). Creatinine kinase (CK- EC 2.7.3.20) activity was determined by other commercial kits and measured by a spectrophotometer at the band of 340 nm. Luteinizing hormone (LH) was measured by SERONO method.

Statistical analysis

Data are presented as means ±SD. Statistical significance of differences between means was calculated by Student's t-test.

Diagnostic validity of the obtained results for discrimination between "on-cycle " and "off-cycle" in AAS users was estimated by the Bays theorem [4]. The theorem was applied for one discrete variable measured in serum and qualified as a positive or negative case by a classical urine test. The 2 x 2 cells gave, therefore, four possible combinations of the test validity: true positives-TP, true negative-TN, false negative-FN, and false positives-FP. Diagnostic sensitivity, specificity, false positive tests, false negative tests, predictive value of positive test, predictive value of negative test and efficiency of the tests have been calculated [4].

Anthropometric measurements

Table 1 illustrates same anthropometric characteristics of the subjects. Statistically significant differences in the body mass and body mass index were observed between both groups of subjects.

Table 1: Characteristics of the body builders using AAS, during "on-cycle" and "off-cycle".

Subjects	No	Age (years)	Body mass (kg)	Height (cm)	BMI kg/m^2	BSA m^2	Years of abuse	Weeks "on" or "off"
"on-cycle"	17	27,0 ±6,7	93,8* ±13,1	175,5 ±5,5	30,2* ±6,7	2,1 ±0,2	2^ 0,1-12	4^ 2-6
"off-cycle"	13	29,0 ±7,3	87,2 ±10,8	174,0 ±4,3	28,7 ±3,2	2,0 ±0,1	3^ 1-12	18^ 4-40

* $p<0,05$; ^- median

The blood and urine samples were taken during 4^{th} week of drugs abusing and during 18 weeks after cessation of the application of the drug. Classification of the subject to group "on-cycle" or "off-cycle" depended on the presence or absence of AAS detected in urine samples.

Results and discussion

Table 2 shows the drugs applied by the body builders, the dosage administrated by themselves, the time of the drugs abuse and substances detected in urine. Some of the body builders declared using a high dosage (from 100 to 200 mg per day) of clenbuterol (Spiropent) as well as the human growth hormone (from 1 to 4 u.m per day, during 3 or 4 months).

Table 2: Anabolic-androgenic steroids used during "on-cycle" in body builders.

No	Age	Declaration of admitted drugs	Substances detected in urine	Duration of abuse	Duration of cycle	Dosage of drugs abused
1	20	Metanabol 5 mg Nerobolil 25 mg Primobolan 100	metandienone nandrolone metenolone	1.5 years	4 weeks	1470 mg 600 mg 2750 mg
2	23	Metanabol 5 mg Retabolil 50 mg Primobolan 100	metandienone nandrolone metenolone	3 years	3 weeks	630 mg 400 mg 1500 mg
3	22	Metanabol 5 mg Primobolan 100 Retabolil 50 mg	metandienone metenolone nandrolone	1,5 years	4 weeks	980 mg 500 mg 2750 mg
4	25	DECA-Durabolin Testosterone	nandrolone T/Et=48.6	2 years	2 weeks	400 mg 400 mg
5	24	Primobolan 100 Metanabol 5 mg	metenolone metandienone	0.5 year	6 weeks	500 mg 840 mg
6	32	Metanabol 5 mg Omnandren 250	metandienone T/Et=141.0	3 years	6 weeks	1260 mg 1500 mg
7	20	Metanabol 5 mg Retabolil 50 mg Testosterone 50	metandienone nandrolone T/Et=92.8	1 year	3 weeks	245 mg 300 mg 300 mg
8	29	Metanabol 5 mg Primobolan 100 Nerobolil 25 mg Omnandren 250 hGH 4 u.m/day	metandienone metenolone nandrolone T/Et= 24.0	2 years	2 weeks	210 mg 250 mg 800 mg 500 mg 360 u.m
9	39	Oxandrolone 2,5 Spiropent 0,02 Testosterone 25 hGH 4 u.m./day	oxandrolone clenbuterol T/Et=13.0	12 years	6 weeks	385 mg 4200 mg 525 mg 168 u.m
10	39	Metanabol 5 mg Primobolan 100	metandienone metenolone	12 years	6 weeks	1260 mg 200 mg
11	29	Ratabolil 50 mg Testosterone hGH 1 - 3 um/day	nandrolone T/Et= 16.4	2 months	5 weeks	500 mg 500 mg 42 u.m
12	25	Metanabol 5 mg Retabolil 50 mg Testosterone 100	metandienone nandrolone T/Et=16.4	2 years	4 weeks	700 mg 350 mg 400 mg
13	31	Metanabol 5 mg	metandienone	4 years	5 weeks	385 mg
14	34	Metanabol 5 mg	metandienone	1 month	6 weeks	840 mg
15	21	no declaration	nandrolone	1 year	off 3 m *	-
16	23	no declaration	nandrolone	2 years	off 3 m*	-
17	39	no declaration	nandrolone	12 years	off 1.5 m*	-

* months

Metandienone had been used most frequently (11 cases in 17 subjects). Nandrolone had been detected in 10 of 17 samples of urine. Three of the body builders strongly denied using any drugs within the period from 6 to 24 weeks before the test, but there were still metabolites of nandrolone existing in their urine samples. They were, therefore, classified as a being "on-cycle".

HDL-C values were below 1 mmol/l in 14 body builders detected positive in the urine test (Tab.3). It should be noted that in 72 analyses of HDL-C in blood samples taken from elite Polish oarsmen no one vale was found below the 1 mmol/l. Serum CK activities were very high. (above 1 000 U/l) in body builders during "on-cycle". The self-application of large doses of β_2-agonists (clenbuterol) and hGH would explain such increase in CK activity. The high CK activity was probably originated in skeletal muscle [2]. Checking up of 92 results of CK activity in resting elite oarsmen we did not find any value exceeding the level of 300 U/l. LH was probably reduced in effect of a negative feedback from exogenous AAS on the hypothalamus and pituitary glands [3]. The "cut off" value for LH below 1 mUI/ml seemed to be a good test for discrimination between "on" and "off" cycle in AAS users [3].

Table 3: High density lipoproteins-cholesterol (HDL-C) with cut off <1.0 mmol/l, creatinine kinase (CK) activity (>300 U/l) and luteinizing hormone (LH) with cut off <1.0 mIU/l in body builders.

Results of urine analyses	No of subjects	HDLC <cut off>		CK activity >cut off<		Serum LH <cut off>		HDLC+CK + Serum LH	
Positive AAS	17	14	3	11	6	13	4	16	1
Negative AAS	13	2	11	3	10	1	12	6	7

Sensitivity of HDL-C test was 82,4% (Tab. 4). The low sensitivity was related to normal concentration of HDL-C in three subjects, whose urine samples exhibited nandrolone (false negative results) [1]. Even after three months of break in drug abusing two body builders still exhibited a low level of HDL-C (false positive results). Efficiency of HDL-C in discrimination between "on" and "off" cycle was 83%.

Table 4: Diagnostic validity of the analysed tests.

	HDL-C <1 mmol/l	CK activity >300 U/l	Serum LH <1,0 mIU/l	HDLC+CK + Serum LH
Sensitivity	82%	65%	77%	94%
Specificity	85%	77%	92%	54%
False positive	15%	23%	8%	46%
False negative	18%	35%	23%	6%
Prediction of positive	88%	79%	93%	73%
Prediction of negative	77%	63%	75%	88%
Efficiency	83%	70%	83%	77%

Sensitivity (65%) and specificity (77%) of CK activities were lower than those observed in HDL-C. For LH, however, the sensitivity was only 76.5% but specificity attained the highest value (92.3%).

The analysis of the three tests considered together gave some advantage by high sensitivity (94%). However, the specificity in this case was relatively low (54%). It seems that the three tests should be assessed as a first step for screening of AAS abuse.

The predictive value of positive tests depends upon the prevalence of AAS abuse in the population under study. In large population of sportsmen where the prevalence of AAS abuse is relatively small (1% - 2%, according to the annual reports of doping control) the predictive value of positive tests will be only about 5%. On the other hand, when these tests would be applied to group of athletes where prevalence of AAS abuse is about 60% - 80% the predictive value of positive result would be 75% - 90% respectively. The practical meaning of the finding is that in 100 positive cases the tests are able to detect up to 90% of AAS users [4]. Finally it can be concluded that determination of HDL-C, CK and LH in blood serum may be useful method for discrimination between "on" versus "off" cycle in AAS users.

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